

**AMENDMENTS TO THE CLAIMS**

1. (Amended) An electrokinetic stirring method for rapid mixing of an initially heterogeneous solution whose motion is dominated by viscous forces, said method comprising an act of:

5 inducing an electrokinetic flow instability (EKI) in said initially heterogeneous solution with an alternating current (A/C) electric field, wherein said EKI, generated within a few seconds after application of said A/C electric field, essentially confined to a mixing chamber, and acting as an active stirring means, quickly produces a randomly fluctuating, three-dimensional fluid flow field enabling said rapid mixing thereby generating a  
10 homogeneous solution from said initially heterogeneous solution.

2. (Original) The method of claim 1, further comprising the acts of:

providing a fluidic network having a plurality of ports including at least two inlet ports and one outlet port, and a plurality of liquid channels connecting said plurality of ports;

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introducing small volume liquid streams into said fluidic network via said inlet ports wherein said liquid streams are characterized as confluent and wherein said confluent liquid streams form said initially heterogeneous solution.

20 3. (Original) The method of claim 2, further comprising the acts of:

positioning two electrodes into ends of said liquid channels wherein said ends also act as inlet and outlet ports for said fluidic network; and

introducing said A/C electric field into said fluidic network via said electrodes.

4. (Original) The method of claim 2, wherein

said A/C electric field is directed axially along one of said liquid channels parallel to a confluent flow direction of said liquid streams.

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5. (Original) The method of claim 2, wherein said liquid channels further comprise at least two side channels with corresponding side channel ports, wherein said fluidic network further comprises a mixing chamber, and wherein either side of said mixing chamber having said side channels connected thereto, said method further comprising the acts of:

10 positioning electrodes into said side channel ports; and

applying said A/C electric field via said electrodes, wherein said A/C electric field is directed along said side channels.

6. (Original) The method of claim 5, further comprising acts of:

15 providing each of said side channels with a high flow resistance, porous, dielectric membrane that mechanically isolates said initially heterogeneous solution, prevents electrolysis bubbles from passing through or otherwise disturbing the liquid in the mixing chamber, and provides an ionic connection allowing passing of said A/C electrical field such that said rapid mixing can be achieved without effects of flow motions and electrolysis gases.

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7. (Original) The method of claim 5, wherein

said liquid streams are advected either electroosmotically or with pressure toward said mixing chamber.

8. (Original) The method of claim 2, wherein  
said rapid mixing is achieved continuously or intermittently where throughput of said liquid  
streams is actuated by either pressure or electroosmotic forces.

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9. (Original) The method of claim 2, wherein  
said liquid streams are advected either electroosmotically with a steady (D/C)  
component simultaneously added to said A/C electric field or by pressure-source means  
including a hydrostatic head, gas-pressurized liquid reservoirs, syringe pumps, or  
10 micropumps.

10. (Original) The method of claim 1, further comprising an act of:  
incorporating electrically conductive, porous, high flow resistance means to prevent  
flow motions and electrolysis gases from affecting said rapid mixing while providing an  
15 electric connection to facilitate said rapid mixing.

11. (Original) The method of claim 1, further comprising an act of:  
pulse modulating between said A/C electric field effecting said EKI and a steady  
(D/C) electric field effecting electroosmotic transport.

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12. (Original) The method of claim 1, further comprising an act of:  
adding a steady (D/C) component simultaneously to said A/C electric field for  
effecting electroosmotic transport.

13. (Original) The method of claim 1, further comprising an act of:

providing at least one pressure-source means for effecting advection, wherein said pressure-source means includes a hydrostatic head, a gas-pressurized liquid reservoir, a syringe pump, or a micropump.

14. (Original) The method of claim 1, wherein

said homogeneous solution is generated from a fixed volume of said initially heterogeneous solution without net flow.

15. (Original) The method of claim 1, wherein

said initially heterogeneous solution comprises low diffusivity species including macromolecules, biological cells, or both.

16. (Original) The method of claim 1, further comprising an act of:

incorporating a monitoring means for analyzing and monitoring performance of said rapid mixing.

17. (Original) An electrokinetic instability (EKI) micromixer, comprising:

a fluidic network having

a mixing chamber;

a plurality of ports including at least two inlet ports, at least two side channel ports, and an outlet port;

a plurality of liquid channels connecting said mixing chamber and said plurality of ports; and

at least two high flow resistance, porous, dielectric membranes; wherein during operation of said EKI micromixer an alternating current (A/C) electric field is applied via said side channel ports for inducing an electrokinetic flow instability (EKI) to effect rapid mixing of an initially heterogeneous solution in said mixing chamber, thereby generating a homogeneous solution from said initially heterogeneous solution.

18. (Original) The EKI micromixer of claim 17, further comprising:

electrically conducting means positioned in said side channel ports for facilitating application of said A/C electric field.

19. (Original) The EKI micromixer of claim 17, wherein

said high flow resistance, porous, dielectric membranes are externally attached to said side channel ports for mechanically isolating fluids in said EKI micromixer to prevent flow motions and electrolysis gases from affecting said rapid mixing while providing an ionic connection allowing passing of said A/C electric field.

20. (Original) The EKI micromixer of claim 17, further comprising:

a modulating means for pulse modulating between an A/C electric field effecting said EKI and a steady (D/C) electric field effecting electroosmotic transport.

21. (Amended) The EKI micromixer of claim 17, further comprising:

a direct current (D/C) source means for providing a steady D/C component that is simultaneously added to said A/C electric field for effecting advection towards said mixing chamber [[by]].

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22. (Original) The EKI micromixer of claim 17, wherein

said rapid mixing has a continuous or intermittent mode driven by either pressure or electroosmotic forces.

10 23. (Original) The EKI micromixer of claim 17, further comprising:

at least one pressure-source means for effecting advection towards said mixing chamber.

24. (Original) The EKI micromixer of claim 23, wherein

15 said at least one pressure-source means includes a hydrostatic head, a gas-pressurized liquid reservoir, a syringe pump, or a micropump.

25. (Original) The EKI micromixer of claim 17, wherein

said homogeneous solution is generated from a fixed volume of said initially  
20 heterogeneous solution without net flow.

26. (Original) The EKI micromixer of claim 17, wherein  
said initially heterogeneous solution comprises low diffusivity species including  
macromolecules, biological cells, or both.

5 27. (Original) The EKI micromixer of claim 17, further comprising:  
an optically accessible means for allowing analyzing and monitoring performance of  
said rapid mixing.

28. (Previously presented) The EKI micromixer of claim 17, wherein  
10 said EKI micromixer characterized as requiring no moving parts is part of a single  
microfluidic chip utilized in a bioanalytical system.

29. (Withdrawn) A method for producing an electrokinetic instability (EKI) micromixer  
capable of utilizing an electrokinetic flow instability for rapid mixing of an initially  
15 heterogeneous solution to generate a homogeneous solution, said method comprising:

wet-etching on a first glass substrate a fluidic network having a mixing chamber, a  
plurality of ports including at least two inlet ports, at least two side channel ports, an outlet  
port, and a plurality of liquid microchannels connecting said mixing chamber and said  
plurality of ports;

20 drilling thru-holes through a second glass substrate, wherein said thru-holes  
correspond to said plurality of ports; and

sealing said fluidic network by thermally bonding said second glass substrate to said  
first glass such that

fluids introduced into said inlet ports can be advected either electroosmotically or with pressure toward said mixing chamber, and

said side channel ports, connected to either side of said mixing chamber, allow for an alternating current (A/C) excitation to induce said EKI in said mixing chamber during operation of said EKI micromixer, wherein said EKI effects said rapid mixing in said mixing chamber.

30. (Withdrawn) The method of claim 29, wherein

said EKI micromixer is an entirely two-dimensional structure, and wherein

10 said fluidic network is etched to a depth ranging from 10 to 5000  $\mu\text{m}$ .

31. (Withdrawn) The method of claim 29, wherein

each liquid channel is characterized as having a width ranging from 10 to 10000  $\mu\text{m}$  and a depth ranging from 10 to 5000  $\mu\text{m}$ .

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32. (Withdrawn) The method of claim 29, wherein

said mixing chamber is characterized as having a volume size ranging from 0.01  $\mu\text{L}$  to 1  $\mu\text{L}$ .

20 33. (Withdrawn) The method of claim 29, wherein

said thru holes are characterized as having diameters ranging from 10 to 5000  $\mu\text{m}$ .

34. (Withdrawn) The method of claim 29, further comprising



attaching high flow resistance, porous, dielectric membranes externally to said side channel ports for mechanically isolating said introduced fluids in said EKI micromixer to prevent flow motions and electrolysis gases from affecting said rapid mixing while providing an ionic connection allowing passing of said A/C excitation.

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35. (Withdrawn) The method of claim 29, further comprising:

incorporating external compression fittings tightly attached to said side channel ports such that high flow resistance, porous, dielectric membranes in said fittings mechanically isolate said introduced liquids in said EKI micromixer while allowing passing of said A/C  
10 excitation.

36. (Withdrawn) The method of claim 29, wherein

said rapid mixing is characterized as having a continuous or intermittent mode driven by either pressure or electroosmotic forces.

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37. (Withdrawn) The method of claim 29, further comprising:

incorporating at least one external pressure-source means for effecting advection towards said mixing chamber, wherein

said pressure-source means includes a hydrostatic head, a gas-pressurized liquid  
20 reservoir, syringe pumps, or any micropumps.

38. (Withdrawn) The method of claim 29, wherein

said homogeneous solution is characterized as having a fixed volume and wherein

said EKI micromixer capable of generating said initially heterogeneous solution without net flow.

39. (Withdrawn) The method of claim 29, wherein

5       said initially heterogeneous solution is characterized as having low diffusivity species including macromolecules, biological cells, or both.

40. (Withdrawn) The method of claim 29, wherein

10       said EKI micromixer is characterized as requiring no moving parts and capable of being easily incorporated into a single microfluidic chip with little or no modification to a standard lithographic mask of said microfluidic chip.